PICOSECOND DETECTION OF BChl-800 AS AN INTERMEDIATE ELECTRON CARRIER BETWEEN SELECTIVELY-EXCITED P_{870} AND BACTERIOPHEOPHYTIN IN *RHODOSPIRILLUM RUBRUM* RELACTION CENTERS

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1. Introduction

The capability of bacteriochlorophyll (BChl) and bacteriopheophytin (Bph) to be reduced was demonstrated in vitro in 1951 [1]. In reaction centers of *Chromatium minutissimum, Chromatium vinosum* and *Rhodopseudomonas viridis* in the presence of reduced complex of ubiquinone and Fe, the excitation of the primary electron donor (P: (BChl *a*)$_2$-870 or (BChl *b*)$_2$-960) results in the electron transfer from cytochrome *c* to Bph with the formation of radical anion, Bph^- [2–4]. Under these conditions the nanosecond flash at 880 nm induces a simultaneous bleaching of the BChl and Bph bands at 600 nm and 545 nm with the formation of the radical anion band at 680 nm [5–6]. These data can indicate the charge separation between P and Bph in ns time domain. Picosecond measurements have also shown that during ~10 ps after flash a simultaneous bleaching of the BChl and Bph bands are observed [6,7]. These results have been interpreted as an indication of the charge separation between P and Bph during 10 ps [8]. However the ps measurements have been carried out upon illumination at 530 nm which induces a bleaching of the pigment bands related to both the charge separation and the excitation of 2 Bph and 4 BChl molecules in the reaction center. Therefore in this work the ps excitation at 880 nm has been used to study the electron transfer reaction in reaction centers. This excitation causes only the formation of the excited state of 2 BChl molecules of P.

The study of photodichroism and circular dichroism of *Rps. viridis* reaction centers in various states have shown that the direct interaction between P and Bph transitions is not observed [3,10,11]. However P and Bph interact with 2 BChl *b* molecules absorbing in the region of 830–850 nm [3,10,11] which corresponds to the 800–810 nm region of absorbance of BChl *a*-containing reaction centers [9]. These 2 BChl molecules have been assumed to be intercalated between P and Bph and can be intermediate electron carriers between P and Bph [3,9–11].

This work demonstrates that during 15 ps excitation at 880 nm the difference absorption spectrum of *Rhodospirillum rubrum* reaction centers shows the formation of P^+ and radical anion of BChl molecule absorbing at 800 nm. Under these conditions a bleaching of Bph bands is not observed. The BChl band at 800 nm recovers into the initial state with the life-time of 35 ± 5 ps. This process is accompanied by the formation of the Bph radical anion. The lifetime of the state $[P^+ Bph^-]$ is 250 ± 50 ps and is determined by the electron transfer from Bph^- to ubiquinone.

2. Materials and methods

Reaction centers were isolated from *Rhs. rubrum* chromatophores by the method in [12], using dodecyldimethylamino-oxide for solubilization. Reaction centers were diluted by 0.05 M carbonate buffer, pH 9.3. The measurements were carried out at 4°C and $E_h \simeq +200$ mV in a 1 mm or 2 mm light path.

The ps absorption measurements were carried out with a set-up described in [13]. A single pulse (with energy of 30 mJ, duration of 30 ps and $\lambda = 1064$ nm) from a mode-locked Nd^{3+}/YAG laser was separated into two beams. The first one generated the ps continuum (in D_2O) which was used as a measuring flash. The second beam after frequency-doubling was passed through the parametric generator [14] to provide a 880 nm excitation flash lasting about 25 ps with energy of 0.5–1.0 mJ. The measurements were carried out in the region of 520–900 nm using the double-beam spectrophotometer. The time resolution of the set-up was ~15 ps. Each point of the spectra and the kinetics was an average of 15–35 measurements.

3. Results and discussion

Figure 1 shows the difference (light – dark) absorption spectra measured at 360 ps, 30 ps and 0 ps (the coincidence of the measuring and exciting flashes) after the 880 nm exciting flashes. Difference spectrum measured at 360 ps mainly corresponds to the formation of P^+ (see [2]) and is characterized by a bleaching of the 870 nm and 600 nm bands, by a blue shift of the 800 nm band and by a developing of the wide bands in the region of 530–580 nm and 670–720 nm. The spectrum also includes a red shift of the Bph band at 750 nm. This shift is caused by the influence of electric field of radical anion of ubiquinone [15,16].

The difference spectrum measured at 30 ps includes the bands corresponding to the formation of P^+ as well as a developing of the wide absorption band at 680 nm and a bleaching of Bph bands at 545 nm and 750 nm. The bands at 545 nm, 750 nm and 680 nm reflect the formation of Bph radical anion which has been observed in reaction centers of *Chr. minutissimum* and *Chr. vinosum* [2,4] and in model systems [8].

The difference spectrum measured at 0 ps involves the bands corresponding to the formation of P^+ as well as a developing of the wide 660 nm band and a bleaching of BChl bands at 600 nm and 800 nm. In the region of Bph bands at 545 nm and 750 nm no bleaching is observed at 0 ps after the 880 nm exciting flashes. The difference absorption bands at 600 nm, 660 nm and 800 nm probably reflect the formation of radical anion of BChl molecule (see [8]) absorbing at 800 nm in the reaction center.

Figure 2A shows the kinetics of absorbance changes at 677 nm, 748 nm, 800 nm and 870 nm. A bleaching of bands at 800 nm and 870 nm as well as a developing of the 790 nm (is not shown) and 660–680 nm bands are observed within the ~15 ps instrumental resolution time. However the kinetics of absorbance changes at 748 nm reflects two processes:

1. During ~15 ps the absorbance increase is observed which is probably related to the formation of the state $[P^+ \cdot \text{BChl-800}^-]$.

2. Then, a bleaching of the 750 nm band occurs which reflects the formation of the state $[P^+ \cdot \text{Bph}^-]$ within ~35 ps (fig.2A).

The formation of the state $[P^+ \cdot \text{Bph}^-]$ is accompanied by the relaxation of the 800 nm band in the initial state, with $\tau \simeq 35 \pm 5$ ps (fig.2A). The 680 nm band disappears with $\tau \simeq 250 \pm 50$ ps (fig.2B). The 30 ps kinetics of the 800 nm band and the 250 ps kinetics of the 680 nm band have been described for *Rps. spheroides* reaction centers upon illumination at 530 nm [6].

Thus, during ~15 ps the electron transfer from P^* with the formation of P^+ is observed as shown in [17]. The electron appears to be extracted from P by BChl-800 which gives a radical anion, BChl-800^-. Then the electron transfer from BChl-800^- to Bph occurs with $\tau \simeq 35$ ps and is accompanied by the formation of Bph^-. During 250 ps Bph^- seems to be oxidized by ubiquinone in agreement with data in [18]. The sequence of the electron transfer in the pigment complex of the reaction center is in agreement with the data on the arrangement and transition interaction of chromophores in reaction centers [3,9–11]:

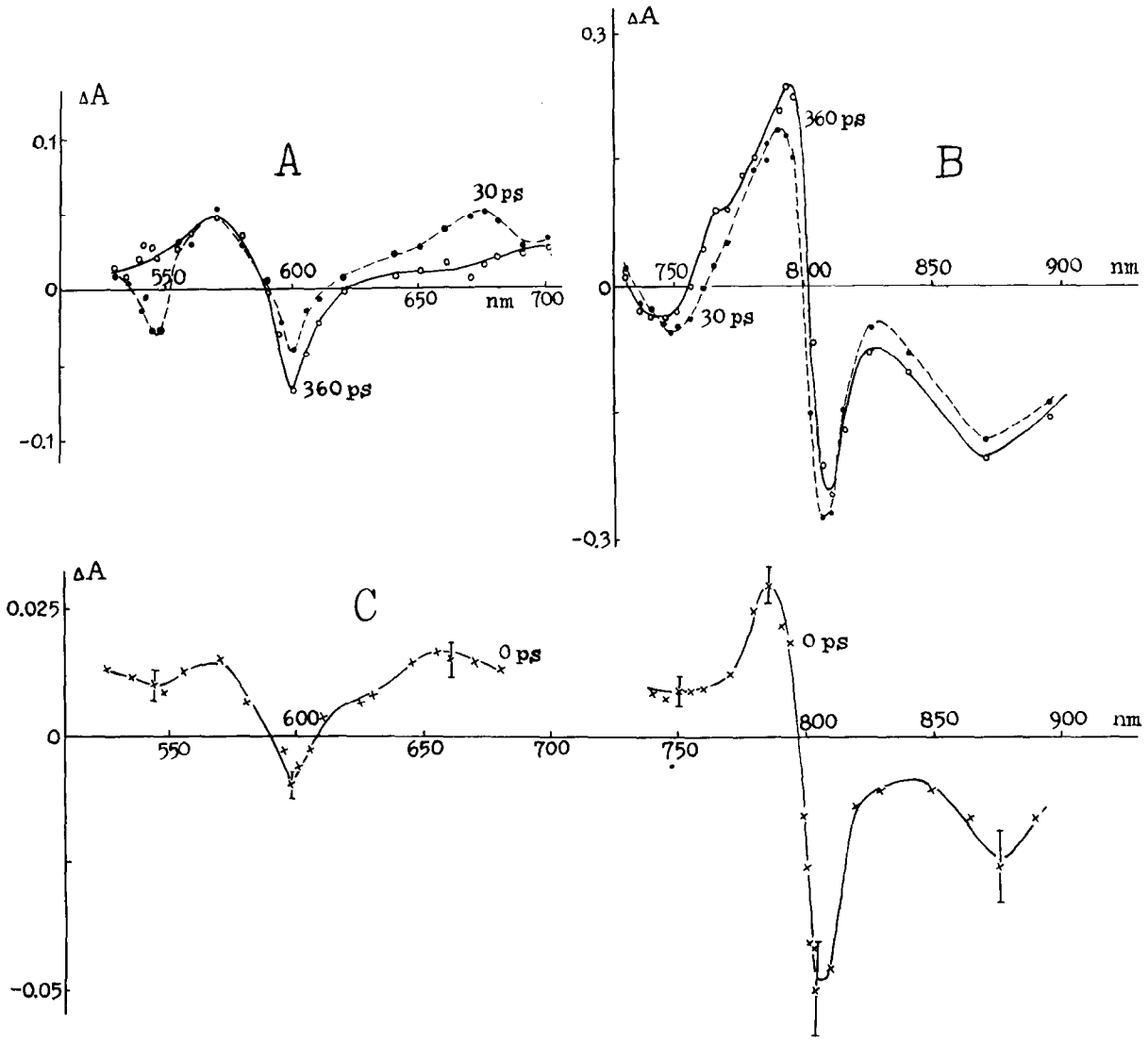
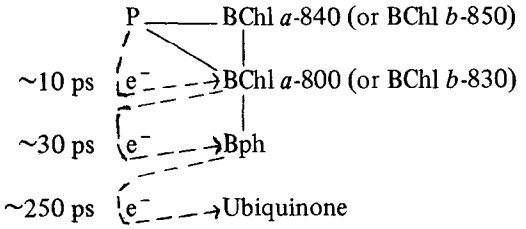


Fig.1. Difference (light – dark) absorption spectrum of reaction centers of *Rhodospirillum rubrum* at 4°C and $E_h \simeq +200$ mV as measured at 360 ps, 30 ps and 0 ps after flashes at 880 nm (duration ~25 ps, energy 0.5–1.0 mJ). The flashes did not saturate the absorbance changes in the sample. The absorbance of the sample at 880 nm was 1 for A and 0.5 for B and C.



where solid lines between the chromophores indicate the interactions of the chromophore transitions, the energies of which are in the region of 100–250 cm^{-1} [11]. The distances between the interacting chromophores are less than 13 Å [11]. The formation of radical anion of ubiquinone only changes the frequency of Bph transition at 750 nm [15,16]. This and EPR

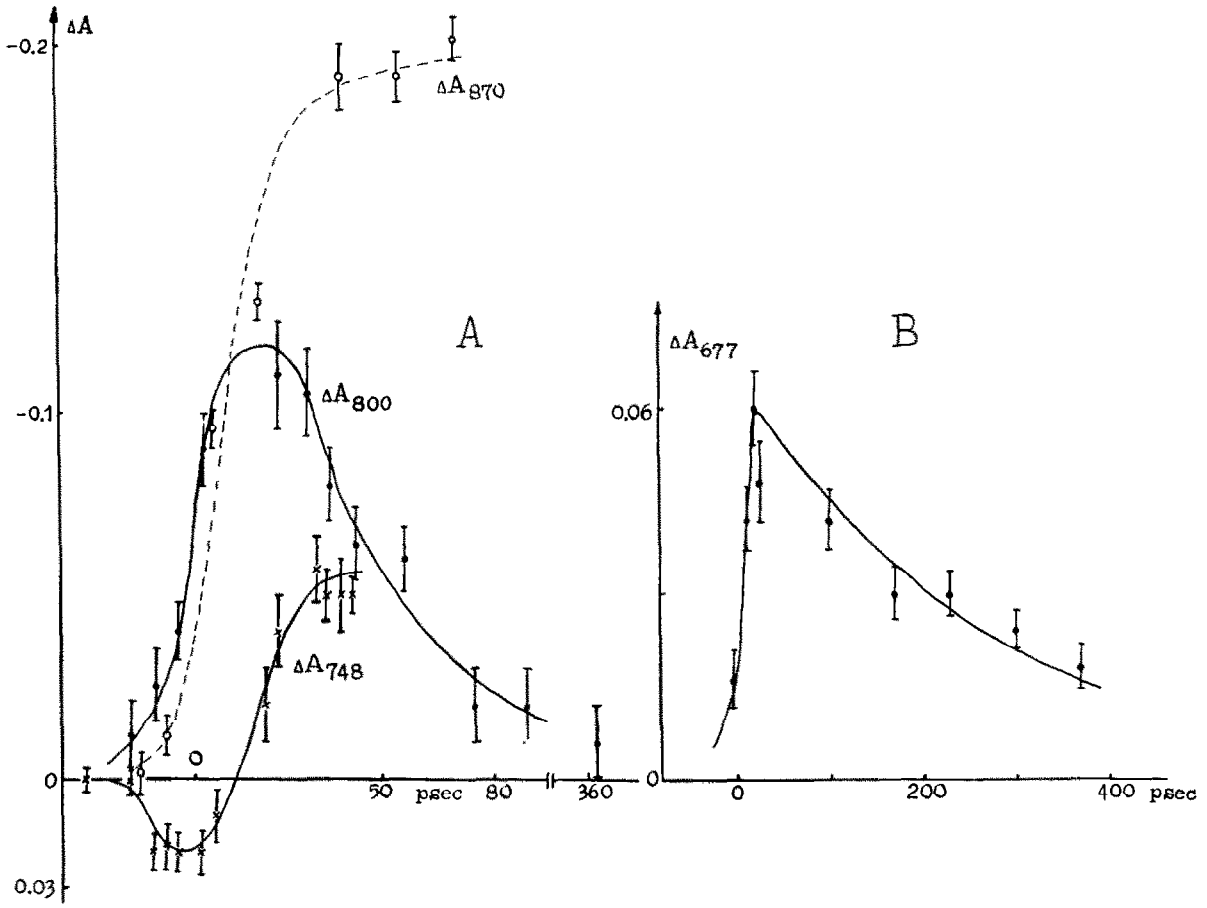


Fig.2. Kinetics of absorbance changes at 870 nm, 800 nm, 748 nm and 677 nm of *Rhs. rubrum* reaction centers at 4°C and $E_h \simeq +200$ mV. The absorbance of the sample at 800 nm was 0.5 for A and 1 for B. The simple consideration shows that kinetics of absorbance changes at 748 nm can be approximated by the sum of two kinetics which are given by:

$$\frac{d[-\Delta A_{748}(t)]}{dt} \sim c_1\,[-\Delta A_{800}(t)] - k_1\,[-\Delta A_{748}(t)] \qquad (1)$$

(absorbance decrease at 748 nm, which is ~0.09 at 50 ps)

$$[\Delta A_{748}(t)] \sim c_2\,[-\Delta A_{870}(t)] \qquad (2)$$

(absorbance increase in the region of 720–760 nm, which is ~0.03 at 748 nm and at 50 ps)

where: c_1 and c_2 are normalizing constants, $k_2 = 1/250$ ps^{-1}

Thus, the kinetics of absorbance changes at 748 nm can reflect the following processes: the charge separation between P and BChl-800 within the ~15 ps instrumental resolution time (absorbance increase), the electron transfer from BChl-800$^-$ to Bph with $\tau \simeq 35$ ps (absorbance decrease) and the electron transfer from Bph$^-$ to ubiquinone with $\tau \simeq 250$ ps (absorbance increase).

measurements [19] show that the complex of ubiquinone–Fe interacts with Bph molecule but does not interact with BChl molecules of the reaction center.

Thus, picosecond measurements show that in the excited pigment complex for the reaction center the electron transfer from P to ubiquinone includes at least 2 intermediate steps. Each step of the electron transfer is accompanied by the loss of the excitation energy [2,3,8,20] and by the steric separation of charges. This results in the stabilization of photoseparated charges in the bacterial reaction center.

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